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IN VITRO MORPHOGENESIS AND MUTATION OF *GLYCINE MAX* L.

by

Thomas Daniel Wilson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

Approved:

_____	_____
Major Professor	Committee Member
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Logan, Utah

1977

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I am very indebted to my family for their continued loyalty.

Thomas Daniel Wilson

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ABSTRACT

In vitro Morphogenesis and Mutation of

Glycine max L.

by

Thomas Daniel Wilson, Master of Science

Utah State University, 1977

Major Professor: Dr. James T. Bowman
Department: Biology

Soybean (*Glycine max* L. c.v. Consoy) tissue cultures were grown in synthetic media containing various concentrations and combinations of minerals, auxins, cytokinins, pH levels, and inorganic growth factors in a systematic procedure to establish optimum media for initiation and maintenance of callus. Further modification of the hormone level was employed to initiate callus morphogenesis. Normally toxic levels of atrazine were established in soybean calli media, and ethyl methanesulfonate (EMS) and X ray-induced mutant calli were selected for atrazine tolerance.

Optimal media for initiation and maintenance of soybean calli were developed and a defined medium was determined that consistently yields root differentiation in nearly 25% of the trials. Atrazine concentrations of 8 mg/l or greater in the medium resulted in 100% fatality of soybean calli; however, approximately 2% of calli treated with X rays or EMS demonstrated resistance to atrazine at

a concentration of 20 mg/l. The implications and potential uses of these developments are discussed.

(25 pages)

SUMMARY

Systematic procedures were developed to produce optimum media for soybean (Glycine max L. c. v. Consoy) callus initiation, maintenance and morphogenesis. A defined medium was developed that consistently yielded root organogenesis from undifferentiated soybean callus tissues in nearly 25% of the trials. Normally toxic levels of atrazine in soybean callus media were determined and ethyl methanesulfonate (EMS) and X-ray-induced mutant calli were selected for resistance to normally lethal levels of atrazine.

INTRODUCTION

Because of the nutritional and economic importance of soybeans (Glycine max L.), a substantial effort has been expended developing methods for regenerating mature soybean plants from undifferentiated tissue. Cultures of soybean cells are readily established from soybean cotyledons, shoots, and roots in liquid and solid medium (e.g., Sargent and King, 1973). The nutrient requirements of soybean cultures have been investigated and a medium consisting of mineral salts, sucrose, vitamins, and 2,4-dichlorophenoxyacetic acid (2-4-D) has been developed (Gamborg et al., 1968). Sporadic root differentiation from callus has been reported (Reid and Galston, 1975) but culture conditions under which root formation consistently occur have not been defined.

Resistance to substantial levels of atrazine has been documented in groundsel (Ryan, 1970; Radosevich and Appleby, 1973) and lambsquarter (Bandeen et al., 1976), and ethyl methanesulfonate (EMS)-induced mutants of wheat and tomatoes have exhibited resistance to herbicides (Pinthus et al., 1972). Recent research by Z. R. Sung (1976) has demonstrated the mutagenic effect of EMS on soybean cells in culture and has provided valuable measurements of the effect of the mutagenic treatment on survival and growth. Sung exposed soybean cells to varying doses of EMS and assayed mutagenesis by recording the appearance of cells resistant to the

drugs 5-fluorouracil at 1 mM and cyclohexamide at 10 µg/ml. Two-hour treatment with 2.5% EMS resulted in 90% mortality but simultaneously increased the frequency of resistant colonies by a factor of 14 over the untreated cells.

In view of these developments, it seemed reasonable to attempt to produce mutant soybean cells capable of growth in normally lethal levels of atrazine.

In this investigation, culture conditions for initiating callus growth for maintaining callus culture and for inducing root differentiation have been defined. Selective levels of atrazine in culture and reasonable conditions for effective mutagenic treatment of soybean callus with EMS and X rays have been determined. Atrazine resistant cells have been produced by EMS and X-ray mutagenic treatments.

MATERIALS AND METHODS

Plant Materials

Seed germination

Seeds of soybean (*Glycine max* L. c.v. Consoy) were surface-sterilized for 1 minute in 95% ethanol followed by 20 minutes in 10% Purex, rinsed three times with sterile distilled water, and placed in petri dishes on filter paper moistened with sterile water. The petri dishes were covered with foil and placed in a constant environment chamber at 24 ^{o}C for 3 days. Leaf tissue surface-sterilized for 3 minutes in 70% ethanol followed by 3 rinses in sterile distilled water was transferred to solid medium for induction of callus growth.

Inoculation of callus

All callus inoculations were performed in a laminar flow cabinet under sterile conditions. Standard inocula were formed by placing calli in a 15-ml centrifuge tube, then macerating and packing them into 3-mm diameter glass tubes. A metal rod was used to extrude the callus onto a tile, and 5-mm segments were cut and placed onto medium in 18 mm x 150 mm culture tubes. After inoculation, the tubes were placed in a constant environment chamber maintained at 24 ^{o}C, 75% humidity, with 1300 lux illumination.

Culture Media

Initiation and maintenance

The conditions required for the initiation and maintenance of soybean callus cultures were determined by systematic procedures similar to those described by de Fossard (1974). The composition of the culture medium is given in Table 1. The scheme involves dividing the compounds essential for growth into four major categories: 1) minerals, 2) auxins, 3) cytokinins, and 4) organics. After buffering media with 2-(N-morpholino)-ethanesulfonic acid (MES) at pH 5.8, each of the four categories were tested at three concentrations (low, L; medium, M; high, H) in all combinations in what is referred to as a broad spectrum experiment. The broad spectrum nomenclature specifies the concentrations of minerals, auxins, cytokinins, and organics in that order. For example, HLMH specifies high minerals, low auxins, medium cytokinins, and high organics. The media were solidified with 1% bacto-agar. After autoclaving, the culture tubes were slanted at a 45^{o} angle. Surface-sterilized leaf tissue was used as the source of initiates in determining optimum nutrient conditions for initiating cell growth. The source of inocula for determining optimum maintenance media was established calli.

pH optima

The medium selected as nutritionally optimum was buffered with MES at pH 5.4 to pH 7.0 in 0.2 increments. Other aliquots were

Table 1. Nutrient components of the broad spectrum experiment (after de Fossard, personal communication)

Constituents	Concentration		
	Low (L)	Medium (M)	High (H)
I. Minerals			
Macronutrients elements (mmol/l)			
NH_4NO_3	5	10	20
KNO_3	-	10	20
KN_2PO_4	0.1	-	-
NaH_2PO_4	-	1	2
KCl	1.9	-	-
$CaCl_2$	1	2	3
$MgSO_4$	0.5	1.5	3
Micronutrients elements (µmol/l)			
H_3BO_3	10	50	150
$MnSO_4$	10	50	100
$ZnSO_4$	1	20	40
$CuSO_4$	0.01	0.1	1.5
Na_2MoO_4	0.01	0.1	1.0
$CoCl_2$	0.1	0.5	1.0
KI	0.5	2.5	5.0
$FeSO_4$	10	50	100
Na_2EDTA	10	50	100
Na_2SO_4	40	450	650
II. Auxins (µmol/l)			
Indole-3-acetic acid (IAA)	0.1	1	10
Indole-3-butyric acid (IBA)	0.1	1	10
Napthalene acetic acid (NAA)	0.1	1	10
Beta-Napthoxy acetic acid (NOA)	0.1	1	10

Table 1. Continued

Constituents	Concentration Low (L)	Medium (M)	High (H)
II. Auxins (continued)			
2,4-Dichlorophenoxy acetic acid (2,4-D)	0.1	1	10
p Chlorophenoxy acetic acid (pCPA)	0.1	1	10
III. Cytokinins (µmol/l)			
Kinetin	0.1	1.0	10
Benzyl amino purine (BAP)	0.1	1.0	10
IV. Organics (mmol/l)			
Sucrose	6.0	60	120
Growth factors (µmol/l)			
Inositol	100	300	600
Nicotinic acid	4	20	40
Pyridoxine HCl	0.6	3	6
Thiamine HCl	0.1	2	40
Biotin	0.04	0.2	1
D-Ca pantothenate	0.2	1.0	50
Riboflavin	0.1	1	10
Ascorbic acid	0.1	1	10
Choline chloride	0.1	1	10
L-Cysteine HCl	10	60	120
Alycine	0.5	5	50

buffered at pH 7.2 with either piperazine-N, N Bis (2 ethanesulfonic acid) 1-Na 1-H_2O (PIPES) or N-2 hydroxyethyl piperazine-N-2 ethanesulfonic acid (HEPES); at pH 7.7 with either HEPES or N-2 hydroxyethyl piperazine-N-2 propanesulfonic acid (HEPPS); at pH 8.2 with either HEPPS or glycyl glycine (GLY-GLY); and at pH 8.95 with either GLY-GLY or cyclohexylaminoethanesulfonic acid (CHES). These were

then inoculated and later scored to determine pH optima and the effects of the various buffers on callus growth.

Induction of morphogenesis

A modification of the broad spectrum experiment was employed to systematically discover and evaluate procedures for inducing differentiation in soybean callus culture. In orthogonal hormone experiments the types and concentrations of the auxins and cytokinins in otherwise optimal medium were varied (Table 2). The media were supplemented with 10g/l casamino acids. These were then inoculated and scored to determine the combination of nutrients and hormones that best stimulates root differentiation.

Table 2. Variable components of the orthogonal hormone experiment

	Concentration			
Auxins (μmol/l)	Null (0)	Low (L^3)	Medium (M^3)	High (H^3)
Indole-3-acetic acid (IAA)	0	0.2	2	20
Indole-3-butyric acid (IBA)	0	0.2	2	20
Naphthalene acetic acid (NAA)	0	0.2	2	20
Cytokinin (μmol/l)	Null (0)	Low (L^Z)	Medium (M^Z)	High (H^Z)
Zeatin	0	0.01	0.1	1.0

Atrazine tolerance

Atrazine at concentrations of 0 mg/l to 22 mg/l in 2.0 mg/l increments was added to medium selected as nutritionally optimum for

maintenance of callus culture. These cultures were then inoculated with standard callus initiates and scored for survival and growth.

Mutagenesis

Ethyl methanesulfonate

Under sterile conditions, soybean calli were cut into 5-mm segments and soaked in a nutritionally optimum liquid medium containing 2.5% EMS. The selection of this concentration of EMS was based on data presented by Sung (1976). After 3 hours incubation in a reciprocating waterbath at 27 ^{o}C and 50 rpm, the calli were placed on a 150-mesh metal sieve and washed several times with fresh sterile medium. The calli were then inoculated onto nutritionally optimum media supplemented with 20 mg/l atrazine. The inoculates were placed at 24 ^{o}C in 75% humidity with 325 lux illumination. Survival and growth were recorded after 13 weeks. Growth of each callus was scaled from 0 to 5; no growth was scored as 0, minimal growth 1, maximum growth 5, and intermediate growth either 2, 3, or 4. The controls followed the same procedures except the calli were inoculated onto media free of atrazine.

X rays

Under sterile conditions soybean calli were prepared for inoculation (macerated and separated into 5-mm long, 3-mm diameter segments) and placed into petri dishes containing 2% agar in sterile distilled water. These petri dishes were exposed to X-ray radiation doses of either 5 kr, 10 kr, or 20 kr (250 kv, 1 mm Al filtration).

The irradiated calli were then transferred onto optimum maintenance media supplemented with either 10 mg/l or 20 mg/l atrazine. The inocula were placed at 24 ^{o}C in 75% humidity with 325 lux illumination. As in the EMS experiment, controls were not exposed to atrazine after irradiation. Scoring proceeded as with the EMS experiments.

RESULTS

Low concentrations of minerals or organics greatly inhibited the initiation of callus from soybean leaf tissue. Growth of initiates proceeded equally well on medium or high concentrations of any of the four basic components of the media. Regardless of the auxin concentration, M_MM medium generally promoted outstanding growth. Although the auxin concentration did not affect callus growth, it did influence the coloration of the callus. A low auxin concentration routinely produced bright green calli; a high concentration, pale green; and a medium concentration, intermediate. MLMM was therefore adopted as the optimum medium for initiation of viable green callus.

Results of the broad spectrum for defining optimal callus maintenance media are given in Tables 3 and 4. Most established calli did not grow well on media containing low concentrations of minerals, cytokinins, or organics. High concentrations of organics also tended to restrict growth. Auxin concentration did not greatly influence the callus growth but did affect the coloration in the same manner as in the initiation experiment. The most rapid growth occurred on MHMM. Growth was characterized by amorphous, friable aggregates. Callus growth occurs rapidly during the first 30 days after transfer with substantial growth continuing for another 60 days. Under both initiation and maintenance conditions, soybean

calli demonstrate superior growth between pH 5.6 and pH 6.2 with maximum growth occurring at pH 5.8. Lesser and more sporadic growth was recorded at higher pH values. Above pH 6.5 the growth rates were minimal and decreased with increasing pH.

Table 3. Broad spectrum experiment. Most and least successful soybean callus maintenance media defined by broad spectrum experiments on soybean. Letters refer to low (L), medium (M), and high (H) concentrations of minerals, auxins, cytokinins, and organics in that order. Mean growth was determined from 5 replications scored from 0 to 5 replications according to callus size. Average growth for 405 calli was 2.2.

Maximum growth		Minimum growth	
Medium	Mean Growth	Medium	Mean Growth
MHMM	4.2	HLLH	0.2
MLMM	4.0	LLLH	0.6
HLHM	3.8	MHLH	0.6
HMLM	3.5	MHLL	1.0
HMHH	3.4	HLML	1.2
HHHM	3.3	LMHH	1.2
HHLM	3.2	LLHH	1.2
HMMM	3.2	MMML	1.3
MLHM	3.2	HLLL	1.4
MLHH	3.0	HLLM	1.4
MMLM	3.0	MMHL	1.4
MHHM	3.0	MHML	1.4
HMMH	3.0		
HMHM	3.0		
HHMM	3.0		
HHMH	3.0		

Morphogenesis

In the hormone experiment involving 16 different auxin and cytokinin combinations, roots were induced on media containing

Table 4. Mean growth on specific concentrations of minerals, auxins, cytokinins, and organics in various combinations

Component	Concentration	Mean growth
Minerals	L	1.7
	M	2.3
	H	2.4
Auxins	L	2.1
	M	2.2
	H	2.2
Cytokinin	L	1.9
	M	2.3
	H	2.3
Organics	L	1.8
	M	2.7
	H	2.0

containing $MH^{3}H^{Z}M$ and $MH^{3}M^{Z}M$. Results of further root initiation experiments concentrating on these successful media are reported in Table 5. Root elongation began about 32 days after inoculation. There were two types of roots, long, thin and brown, and short, thick and white. The majority of the roots were negatively geotropic and grew into the agar. A few roots grew along the sides of the culture tube and above the agar surface. Some branched root systems developed. As many as 12 roots per callus were observed, with six roots per callus being the average. The bulk of the rooting occurred in the second month of passage. Beyond 60 days only one undifferentiated callus developed roots. Although dark green meristamatic buds did appear, no shoots were observed. After

90 days the differentiated calli were transferred to MOOM medium. No further differentiation occurred.

Table 5. Soybean callus root morphogenesis in media developed by hormone experiment

Medium	Replications	Number of calli that developed roots	Percent of calli that developed roots
$MH^{3}{}_{H}{}^{Z}{}_{M}$	59	5	8
$MH^{3}{}_{M}{}^{Z}{}_{M}$	70	16	23

Atrazine Tolerance

Table 6 illustrates the tolerance of soybean calli to various levels of atrazine presented in MLMM. Calli grown in media containing 2 mg/l atrazine exhibited no signs of growth inhibition. On the average, growth was slightly superior to that of the controls. In media supplemented with 4 mg/l atrazine, 62% of the calli inoculated grew. Their total mass after 135 days in culture was approximately half that of the controls. The calli were light green and friable similar to the controls. Only 25% of the calli treated with 6 mg/l atrazine grew. Lacking vigor, these calli were light brown, friable and reduced in size. Calli subjected to higher concentrations than 6 mg/l atrazine failed to survive. They turned dark brown and became inviable within 21 days after inoculation.

Table 6. Natural atrazine tolerance levels of soybean calli defined by supplemention. Basal medium MHMM.

mg/l Atrazine	Replications	Number of calli that demonstrated growth	Percent of calli that demonstrated growth
0	16	16	100
2	16	16	100
4	16	10	62
6	16	4	25
8	16	0	0
10	16	0	0
12	16	0	0
14	16	0	0
16	16	0	0
18	16	0	0
20	16	0	0
22	16	0	0

Mutagenesis

Results of subjecting calli to mutagenic agents and then transferring them onto media containing normally lethal levels of atrazine are reported in Table 7. Thirty-two percent of the EMS controls demonstrated growth. Growth rates were sluggish at first (only one callus showed growth during the first 25 days after transfer) but later dramatically increased. The surviving calli were not uniform in color, but many possessed speckled areas consisting of dark brown and greens or pale browns and yellows. Many areas of the control calli were nearly black and appeared to be inviable.

Table 7. Survival frequency of soybean calli in normally toxic levels of atrazine after treatment with mutagenic agents. Basal medium MHMM.

Mutagenic treatment	Atrazine concentration in medium	Replications	Number of calli demonstrating growth	Percent of calli demonstrating growth
2.5% EMS for 3 hrs	0 mg/l	31	13	32
2.5% MES for 3 hrs	20 mg/l	144	3	2
X ray 5 kr	0 mg/l	10	10	100
X ray 5 kr	20 mg/l	80	2	2
X ray 10 kr	0 mg/l	9	7	78
X ray 10 kr	20 mg/l	80	1	1
X ray 5 kr	0 mg/l	6	5	83
X ray 5 kr	10 mg/l	60	19	32
X ray 10 kr	0 mg/l	7	7	100
X ray 10 kr	10 mg/l	58	28	48
X ray 20 kr	0 mg/l	7	7	100
X ray 20 kr	10 mg/l	47	22	47

Only 2% of calli treated with EMS and transferred to 20 mg/l atrazine survived. Within 21 days of inoculation all the calli turned brown. Fifty days after transfer a green area extended from a single brown callus and growth proceeded very rapidly. Another callus began growth in the same manner 82 days after transfer. These two growing calli were very viable and lacked the spectrum of dark areas exhibited by the controls.

Of all the X ray control calli, 92% demonstrated growth. The 5-kr treated calli grew faster and were greener than the cultures exposed to 10 kr and 20 kr doses. Two percent of the 5 kr and 1%

of the 10 kr exposed calli grew in 20 mg/l atrazine. These calli were green and friable. Thirty-two percent, 48% and 47% of the cultures exposed to X-ray doses of 5 kr, 10 kr, and 20 kr, respectively, grew in media supplemented with 10 mg/l atrazine. As in the calli treated with EMS, the initial growth rate of viable X-ray-treated calli was slow but subsequently increased rapidly.

DISCUSSION

Because the broad spectrum systematically examines many different combinations of minerals, auxins, cytokinins, and organics, it was possible to approach optimum media for initiation and maintenance of soybean callus. It also exposed particularly undesirable concentrations and combinations of the four major groups of nutritional components. In addition, this experiment greatly facilitated observing the relationships between concentration and composition of the media and the physical properties of the calli.

In the sense of the broad spectrum procedure, the optimum media for initiation and maintenance of soybean callus are MLMM and MHMM, respectively. Low concentrations of minerals or organics consistently produced poor callus growth. In general, medium to high concentrations of minerals, cytokinins and organics produced significantly larger calli.

Auxin concentration did not substantially affect callus growth but it did determine callus color. Vivid green callus resulted from exposure to low concentrations of auxins, whereas high concentrations produced pale green callus. This phenomenon suggests that auxins are involved in the regulation of photosynthesis, probably by controlling the formation of pigments. This hypothesis is currently being investigated in our laboratory.

Comparison of the composition of MHMM with B5 (Gamborg et al., 1968), the most widely used soybean tissue culture medium, reveals similar concentrations of minerals and sucrose, and major differences in the type and concentration of auxin, cytokinin and growth factors. B5 contains only one auxin (2,4-D) at a concentration of approximately 1 mM/l and no cytokinin. MHMM contains six auxins (IAA, IBS, NAA, NOA, 2,4-D and pCPA) at a concentration of 10 mM/l each and two cytokinins (Kinetin and BAP) at 1 mM/l each. Additionally, MHMM includes several growth factors that are not found in B5 (see Table 1).

Superior callus growth occurred at a pH of 5.6 to 6.2 with maximum growth achieved at pH 5.8. Very limited growth occurred at pH 8.95. It is clear that the pH range for soybean callus growth is broad. In the pH experiments, six different buffers were used separately to adjust the media to various overlapping pH levels. Different buffers at the same pH levels did not affect callus growth.

Soybean callus which had not been exposed to mutagens tolerated 2 mg/l atrazine without any decrease in growth. At concentrations greater than 6 mg/l, the callus died. Callus grown in 2 mg/l atrazine actually showed growth slightly superior to that of the controls. This is consistent with the results of an investigation by Bush and Ries (1974) in which the effects of sub-herbicidal application of atrazine on red kidney bean was reported. Atrazine stimulated an increase in fresh weight during the period of

cell elongation and this increase was due to an increase in protein synthesis. Penner and Early (1972) found that atrazine applied to soybeans enhanced RNA synthesis. The increased growth rate of calli exposed to low levels of atrazine could be explained by these previous reports.

The orthogonal hormone experiment further refined the concentrations of auxins and cytokinins that facilitate the differentiation of soybean calli. Results from these experiments led to the development of a medium ($MH^{3}M^{Z}M$) that produced soybean roots from undifferentiated callus in 23% of the trials (see Table 4). To date, this medium provides the most consistent soybean callus differentiation reported. The only modifications in this medium were the hormone levels. This demonstrates that changes in the ratio of auxin to cytokinin in the nutrient media are decisive in determining whether differentiation is initiated or not. The rooting medium, $MH^{3}M^{Z}M$, contains a very high auxin to cytokinin ratio. These results are in accordance with the findings of Skoog and Miller (1957) in their classic paper on plant hormone regulation of organ formation in tobacco tissue culture. They concluded a high auxin to cytokinin ratio produced roots and inhibited the production of shoots. Rooted soybean calli were transferred to MOOM medium containing no auxins or cytokinins. Neither growth nor additional differentiation was observed. This indicates that rooted soybean calli do not produce minimal growth levels of hormones.

Naturally-occurring resistance to high levels of atrazine have been reported (Ryan, 1970; Radosevich and Appleby, 1973; and Bandeen et al., 1976) in several plants. Resistance to herbicides has been induced in cultivars of wheat and tomatoes by treatment with EMS (Pinthus et al., 1972). After soaking the tomato and wheat seeds in EMS, they were planted in loamy sandy soil containing 40 ppm diphenamid (N, N-dimethyl 2,2-diphenylacetamide) and 1 ppm terbutryn (2-tert-butylamino-4-ethylamino-6-methylthio-5-traizine), respectively. Approximately 1.0% of the seeds developed herbicidal resistance and germinated into plants. There was, therefore, reason to expect that increased herbicidal resistance might be obtained by selection from mutagenically-treated populations of soybean cells. This expectation was strengthened by the fact that several mutant cell strains have been established and propagated in tissue culture for several years (e.g., Widholm, 1974).

The current study developed mutants of soybean callus that are resistant to high levels of atrazine. As previously indicated, soybean calli are unable to survive in medium containing greater than 6 mg/l atrazine. Of the mutagenically-treated soybean calli, 2.0% survived in media containing 20 mg/l atrazine, greater than three times the normally toxic level, and 42% survived in medium containing 10 mg/l of atrazine. These experiments prove that resistance to normally toxic levels of herbicides is within the genetic repertoire of soybeans.

Callus plugs exposed to EMS turned brown and a vast majority were inviable. The EMS killed a significant number of callus cells within three weeks of treatment. X-ray-treated cells remained green. The radiation apparently disrupted cytokinesis but did not drastically affect cell metabolism. Larger X-ray doses resulted in slower growth. As expected, if true resistance were induced, a larger percentage of calli exposed to 10 kr or 20 kr of X ray demonstrated resistance to 10 mg/l atrazine than did calli exposed to 5 kr. Higher radiation doses did not affect the survival rate of control calli but did diminish their growth rate. These results indicate, as expected, that higher X-ray dosages cause a greater number of mutations. Both EMS and X-ray treatments yield approximately the same number of atrazine-resistant calli.

Mutagenesis of soybean callus has some distinct advantages over similar treatment of single cells in suspension cultures. Cells lacking resistance to the selective element can act as nurse cells and speed growth of resistant cells. Some plants do not readily lend themselves to single cell isolation and subsequent growth. In these cases, mutagenesis would have to be induced in callus tissue.

The development of a medium that consistently produces relatively high frequencies of root differentiation indicates that total regeneration of soybean should be possible and that present recalcitrance could be eliminated by the proper concentration and combination of nutrients and growth factors. If the current

philosophy (i.e., Steward et al., 1970) that each cell contains all the genetic information needed for the entire plant and that all cells of linear descent will retain this information is accurate, soybean plants resistant to high levels of atrazine can be produced. The prospects of developing soybean cells resistant to normally toxic levels of metal ions, pH, and salinity with the same procedure are promising. Thus potentially valuable cultivars of soybean can be developed from cells that have undergone mutagenesis and selection for advantageous traits.

LITERATURE CITED

Bandeen, J. D., P. C. Bhowmik, and K. N. Jensen. 1976. Two strains of lambsquarters with differing tolerance to atrazine. Weed Sci. Soc. Amer. Abstract 150.

Bush, P. B. and S. K. Ries. 1974. Effect of atrazine on elongation of the embryonic axis of red kidney bean. Weed Sci. 22: 227-229.

de Fossard, R. A. 1974. The broad spectrum tissue culture experiment. Personal communication.

Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exptl. Cell Res. 50:151-158.

Penner, D. and R. W. Early. 1972. Effect of atrazine on chromatin activity in corn and soybean. Weed Sci. 20:367-370.

Pinthus, M. J., Y. Eshel, and Y. Shchori. 1972. Field and vegetable crop mutants with increased resistance to herbicides. Science 177:715-716.

Radosevich, S. R. and A. P. Appleby. 1973. Relative susceptibility of two common groundsel (Senecio vulgaris L.) biotypes to six triazines. Agronomy J. 65:553-555.

Reid, R. K. and A. W. Galstron. 1975. Experiments on the isolation and cultivation of protoplasts and calli of agriculturally important plants. II. Soybean (Glycine max L.). Biochem. Physiol. Pflanzen 168:S. 473-482.

Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614-616.

Sargent, P. S. and J. King. 1973. Investigations of growth-promoting factors in conditioned soybean root cell and in liquid medium in which they grow. Can. J. Bot. 52:1747.

Skoog, F. and C. O. Miller. 1957. Chemical regulation of growth and organ differentiation in plant tissue cultures in vitro. Symp. Soc. Exp. Biol. 11:118-130.

Steward, F. C., P. V. Ammirato, and M. O. Mapes. 1970. Growth and development of totipotent cells; some problems, procedures, and perspectives. Ann. Bot. 34:761-787.

Sung, Z. R. 1976. Mutagenesis of cultured plant cells. Genetics 81:51-57.

Widholm, J. M. 1974. Selection of characteristics of biochemical mutants of cultured plant cells, pp. 287-299. In H. E. Street (Ed.). Tissue culture and plant science. Academic Press, New York.

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